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PRINCIPAL INVESTIGATOR: Lydia Lee Sohn, Ph.D.

CONTRACTING ORGANIZATION: The University of California
Berkeley, CA 94704

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Lydia Lee Sohn

E-Mail: sohn@me.berkeley.edu

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14. ABSTRACT

We report the achievements of the second year of our project. These achievements include development of a high-throughput platform consisting of an array of microposts of different size to investigate the effect of stiffness range on breast-cancer phenotype (given that tumors are heterogeneous in terms of stiffness). We are currently focusing on the MCF-7 a-CD271 phenotype since it has been recently reported to be a candidate breast CaSCs. Through immunostaining, we have identified that phenotype with our MCF-7 cell line. We intend to incorporate microfluidic channels that will deliver specific chemical cues to cells growing on the micropost arrays to best mimic the tumor microenvironment. We will also use our super-resolution microscope developed in Year 1 to image the cells growing to determine spatial pattern changes of surface receptors.

15. SUBJECT TERMS
Stochastic Optical Reconstruction Microscopy (STORM), micropoast arrays, stiffness, a-CD271

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Introduction

In order to increase our understanding of cancer stem cells and their role in cancer progression and metastasis, our overarching goal has been to study *how the cellular microenvironment maintains and controls this cellular phenotype*. To realize this goal, we had proposed to use 3D super-resolution microscopy to visualize how individual breast CaSCs and tumor cells interact with each other and their microenvironment. In Year 1 of this project, we completed the construction of our super-resolution microscope, which now has the capabilities to perform both 2D and 3D multi-color Stochastic Optical Reconstruction Microscopy (STORM) and Photoactivated Localization Microscopy (PALM). We demonstrated our ability to image specific receptors on the surface of MCF-7 breast ductal carcinoma cells. In Year 2, we focused on ***Specific Aim 2: To fabricate small controllable microenvironments, in which we can simultaneously culture small groups of cells and monitor their response to external perturbation***. As we report below, we have examined the effects of microenvironments on breast cancer by creating arrays of polydimethlysiloxane (PDMS) microposts of different stiffness and sizes and seeded them with MCF-7 cells. We manipulated our micropost microenvironments to study the effects of microenvironment on cell lineage, namely the basal-luminal differentiation of MCF-7s.

Body

Specific Aim 2: To fabricate small controllable microenvironments, in which we can simultaneously culture small groups of cells and monitor their response to external perturbation

Our initial plan to create the small controllable microenvironments had been to use an appropriate composition of extracellular proteins and proteoglycans (e.g. collagen gels, matrigel, hyaluronic acid gels, and polyacrylamide gels functionalized with various ECM proteins) in a microfluidic reservoir. We would alter dynamically the mechanical properties of this microfluidic culture environment either through the application of stress or strain via mechanical actuators. Given the complexity of the system and the number of parameters involved, we chose to begin this Specific Aim by creating tunable micropost arrays to study breast cancer cell microenvironments. These arrays are rapidly and easily fabricated, due to an uncomplicated geometry and simple material selection (polydimethylsiloxane – PDMS). Critically, simple fluid flow profiles can be used, with gradient profiles created using the easily altered aspect ratio and stiffness of the microposts. Advantages of our tunable micropost arrays over traditional two-dimensional tissue-culture polystyrene (TCPS) include improvements in microenvironment accuracy and throughput through control of material properties e.g. stiffness and array size/density.

The fabrication process of our tunable micropost arrays relies on standard soft lithographic techniques. Using AutoCAD, we create a mask and use soft lithography to create PDMS (Sylgard 184) microposts with a given aspect ratio and stiffness (typically 2500-5000Pa for breast tumor conditions[1]).

These micropost arrays are highly tunable because altering the stiffness and array size/density is merely dependent on the ratio of PDMS to curing agent and the dimensions of the array, respectively. We chose to create tunable micropost arrays in order to study how the variation of the microenvironment properties using aspect ratio and stiffness affects breast cancer cell differentiation and lineage (basal vs. luminal).

PDMS microposts were created by first constructing silicon master molds using Shipley 1813 positive photoresist. By varying the spin-speed, we are able to control the height of the resulting microposts. PDMS was poured over the master and cured at 80^{o}C for 2 hours. Atomic Force Microscopy (AFM) was used to characterize the height and shape of these microposts (Figure 1).

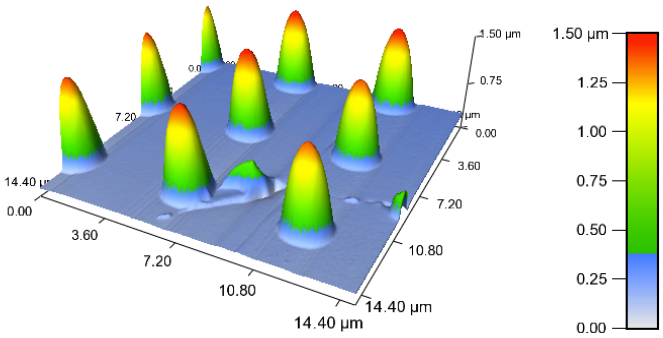


***Figure 1**: AFM was used to characterize micropost size and shape after soft lithography. Based on the above array pattern, we demonstrate an easily tunable micropost array using soft lithographic tools. AFM images were taken using a Veeco Si_3N_4 cantilever, with imaging done in AC mode in air. The sample tested contained 3µm diameter PDMS microposts with a 6µm lattice constant.*

MCF-7 breast ductal carcinoma cells were successfully cultured on our PDMS micropost arrays for over a week in DMEM (Gibco) + 10% fetal bovine serum (Gibco) + 1% penicillin/streptomycin (Invitrogen) (Figure 2). The devices are optically transparent so further analysis such as bright field imaging and immunostaining are facile.

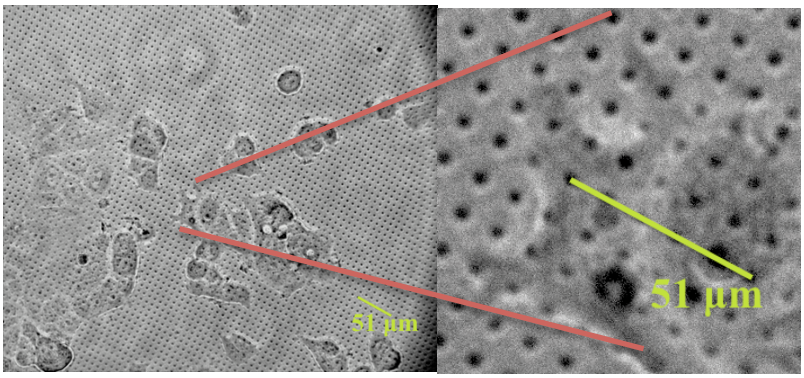


***Figure 2**: Micropost array with MCF-7 breast ductal carcinoma cells plated. Image was taken following one day of culturing on the micropost array. Generous cell spreading and morphology indicate normal cell growth and biocompatibility of our device.*

Based on these initial results, we have created a high-throughput analysis system (Figure 3) that would allow us to investigate the effect of stiffness range on phenotype (given that tumors are heterogeneous in terms of stiffness). We are currently focusing on the MCF-7 α-CD271 phenotype since it has been recently reported to be a candidate breast CaSCs[2]. Through immunostaining, we have identified that phenotype with our MCF-7 cell line (Figure 4).

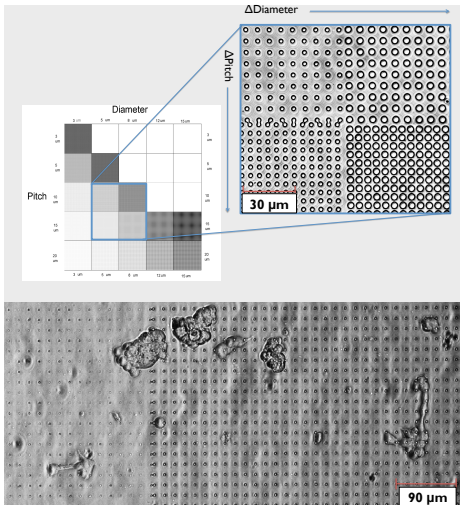


***Figure 3**: (**Top**) In order to test multiple stiffness conditions on a single, high-throughput chip, we have developed a "gradient" micropost array. (**Bottom**) Cell spreading and morphological changes are observed between areas of differing apparent modulus following cell seeding.*

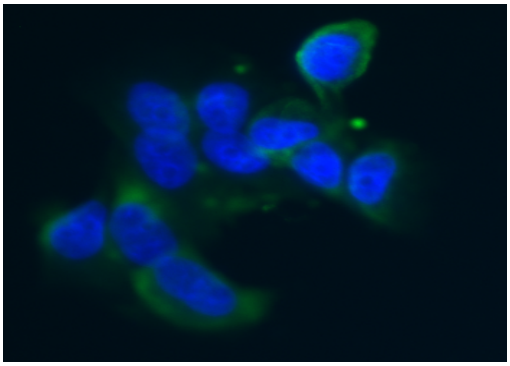


***Figure 4**: MCF-7 breast ductal carcinoma cells stained with an α-CD271 antibody (Abcam p75 NGF). The α-CD271 marker has recently been reported as a stem-like marker for pre-tumorigenic cells.*

Key Research Accomplishments

•Developed a high-throuput platform to investigate the role of stiffness in supporting specific breast-cancer phenotypes

•Identified the α-CD271 phenotype in the MCF-7 cell line that has been reported to be stem-cell like. The rarity of this phenotype in the cell line is <1%.

•Begun to investigate what stiffness supports the α-CD271 phenotype

Reportable Outcomes

Meeting Abstracts

1. Anand Kesavaraju, Bo Qing, Eric Jabart, Marc LaBarge, and Lydia L. Sohn, "Micropost microenvironments for studying luminal-basal lineage commitment of breast-cancer cells", Oral Presentation, American Physical Society Annual March Meeting, Baltimore, MD, March 2013.
2. Anand Kesavaraju, Bo Qing, Eric Jabart, and Lydia L. Sohn, "Tunable Micropost Arrays for Studying Breast Cancer Microenvironments", Poster presentation, 7th International Conference on Microtechnologies in Medicine and Biology, Marina Del Ray, CA, April 10-12, 2013.

List of Personnel Receiving Pay from Research Effort

1. Lydia L. Sohn, PI
2. Matthew R. Chapman, Graduate Student Researcher
3. Anand Kesavarau, Staff Research Scientist in Sohn Laboratory

Additional Personnel on Project

1. Eric Jabart, Graduate Student Researcher
2. Bo Qing, Undergraduate Student Researcher

Conclusion

We continue to make progress on our project. We focused on Specific Aim 2, which is centered on developing a platform to recreate the microenvironment that supports breast-cancer stem cells (α-CD271 phenotype). Our plan in the next year is to integrate the high-throughput post arrays with microfluidic channels that could deliver chemical cues to the growing cells. Furthermore, we will image the cells grown on the post arrays with our super-resolution microscope to track the changes in the spatial distribution of specific receptors. We have hired a postdoctoral fellow—Mustafa Mir (EECS Ph.D., University of Illinois, Urbana-Champagne)—who is an expert in super-resolution microscopy and who will perform such imaging. Mir will begin his appointment on July 1, 2013.

References

1. Paszek, M. J. *et al*. "Tensional homeostasis and the malignant phenotype." *Cancer Cell* 8, 241–254 (2005).
2. Kim, J. *et al.* "Tumor initiating but differentiated luminal-like breast cancer cells are highly invasive in the absence of basal-like activity, PNAS doi: 10.1073/pnas.1203203109

Appendix

Abstract for the American Physical Society Annual March Meeting, Baltimore, MD March 2013

Session T45: Focus Session: Physics of Cancer 1
9:24-9:36 AM, Thursday, March 21, 2013

Abstract: Y45.00006 : Micropost microenvironments for studying luminal-basal lineage commitment of breast cancer cells

Anand Kesavaraju, Bo Qing, Eric Jabart
(Department of Bioengineering, University of California, Berkeley)
Marc LaBarge
(Lawrence Berkeley National Laboratory)
Lydia L. Sohn
(Department of Mechanical Engineering, University of California, Berkeley)

MCF-7 breast cancer cells were plated onto polydimethylsiloxane (PDMS) microposts in order to examine the effects of the microenvironment on cell lineage. Different stiffnesses and sizes of the microposts are postulated to impact cell surface marker expression levels. We will provide preliminary results analyzing CD271 and focal adhesion markers such as vinculin. 3D shear flow will also be applied to the microposts to study how external mechanical stimuli affect cancer cells within their microenvironment.

Poster for the 7th International Conference on Microtechnologies in Medicine and Biology, Marina Del Ray, CA, April 10-12, 2013

Poster: Tunable Micropost Arrays for Studying Breast Cancer Microenvironments

Anand Kesavaraju, Bo Qing, Eric Jabart
(Department of Bioengineering, University of California, Berkeley)
Lydia L. Sohn
(Department of Mechanical Engineering, University of California, Berkeley)

Please see following page for the full poster.

TUNABLE MICROPOST ARRAYS FOR STUDYING BREAST CANCER MICROENVIRONMENTS

Anand Kesavaraju[1], Eric Jabart[1], Bo Qing[1], Lydia Sohn[2]
[1]Department of Bioengineering, University of California, Berkeley, USA
[2]Department of Mechanical Engineering, University of California, Berkeley, USA

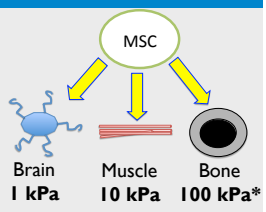
F2P15

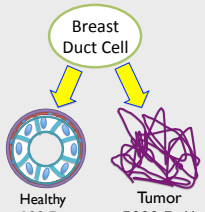
ABSTRACT

Breast cellular microenvironments play a key role in maintaining healthy tissue and disregulation can lead to tumorigenesis. In order to examine the effects of the microenvironment on breast cancer, we created arrays of polydimethylsiloxane (PDMS) microposts of different stiffness and seeded them with MCF-7 breast ductal carcinoma cells. We manipulated our micropost arrays to study the effects of microenvironment on cell lineage, namely the basal-luminal differentiation of MCF-7s. Differentiation of MCF-7s can be monitored optically by morphological analysis and via surface marker expression.

MICROENVIRONMENT BIASES CELL FATE

- Mesenchymal Stem Cell (MSC) differentiation can be biased by the **stiffness** of the substrate

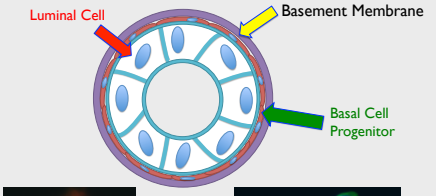


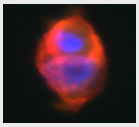


- Similarly, tumorigenesis can be influenced by the **stiffness** of the substrate

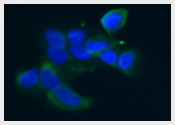
*DOI: 10.1016/j.cell.2006.06.044
**DOI: 10.1016/j.ccr.2005.08.010

MICROENVIRONMENT AFFECTS BREAST CANCER LINEAGE





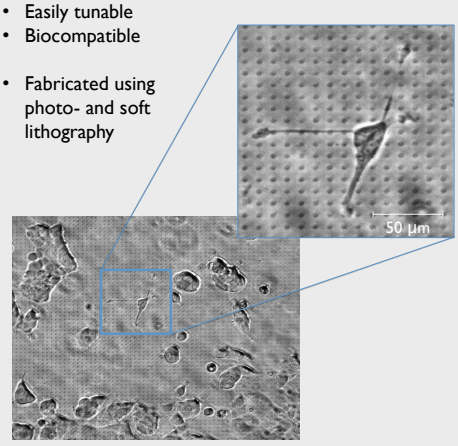
MCF-7 α–MUC1

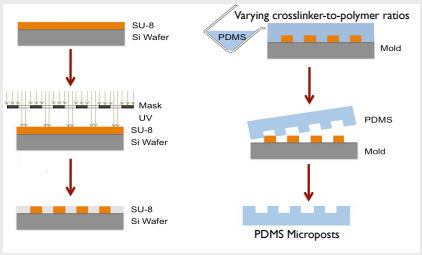


MCF-7 α–CD271

NOVEL MICROPOST PLATORM

- Easily tunable
- Biocompatible
- Fabricated using photo- and soft lithography





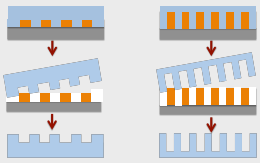
CONFERRING BULK AND APPARENT MODULI

Bulk Modulus

- Material characteristics

Apparent Modulus

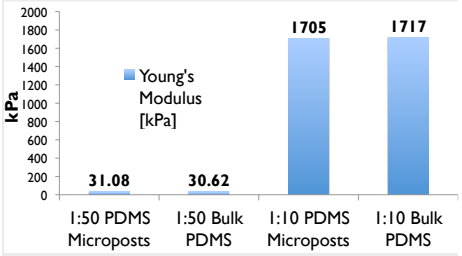
- Pitch
- Aspect ratio



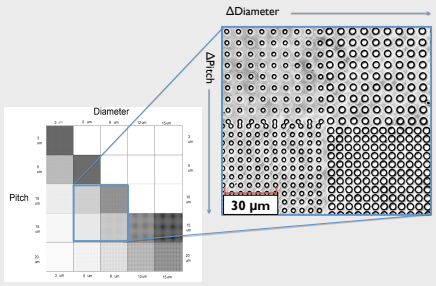
STIFFNESS AS A KEY VARIABLE

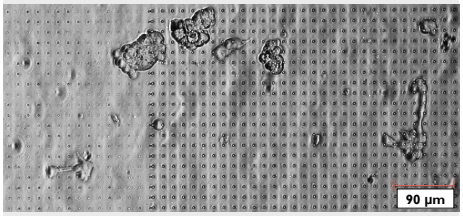
Healthy tissue 100-2400 Pa

Tumor tissue 2500 - 5000 Pa



HIGH THROUGHPUT ANALYSIS





3 µm diameter microposts
15 µm pitch

5 µm diameter microposts
15 µm pitch

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